Reference *Biol. Bull.*, **151**: 386–397. (October, 1976)

THE CHEMICAL ECOLOGY OF *BIOMPHALARIA GLABRATA*: THE EFFECTS OF AMMONIA ON THE GROWTH RATE OF JUVENILE SNAILS

J. D. THOMAS, M. POWLES AND R. LODGE

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QH, Sussex, England, U. K.

The growth and natality rates of *Biomphalaria glabrata* (Say) may be enhanced, in closed systems, by decreasing the volume or by increasing snail numbers or conditioning time to optimum thresholds. Further increases in snail numbers or a decrease in the volume per snail or in conditioning time may result in a decrease in growth and natality rates of the snail (Thomas, 1973). The environmental factors which may be involved in causing the negative feedback effects have been discussed by Thomas (1973) and include ammonia which is the main excretory product of these snails. It is well known that ammonia may be toxic to certain aquatic animals (Kawomoto, 1961; Ball, 1967; Spotte, 1970). The purpose of the work described in the present paper is to determine the extent to which ammonia can influence the growth and survival rates of the snails and thus provide the basis for a negative feedback mechanism.

Materials and Methods

The methods used for maintaining the stock cultures of the Venezuelan, albino strain of *B. glabrata* used in these experiments have been described by Thomas and Benjamin (1974). At the commencement of the experiment they had an initial weight of approximately 20 mg. They were maintained at a temperature of 26 ± 1° C with a photoperiod of 12 L: 12 D in an environmental unit described by Thomas and Benjamin (1974). They were fed on standard daily rations of washed lettuce discs or 3% Bemax made up in agar, provided in excess of requirements. Uneaten remains were removed each day. Two kinds of media made up from deionized water were used in the experiments: standard snail water [SSW(1)], with the following composition in millimoles per liter, Ca 2.0, Mg 0.13, Na 0.63, K 0.086, Cl 0.63, HCO_3 4.037, SO_4 0.13, NO_3 0.049; and SSW(2), -Ca 2.0, Mg 0.13, Na 0.63, K 0.086, Cl 4.0, HCO_3 0.67, SO_4 0.13 and NO_3 0.049.

In most of the experiments ammonia was added in the form of NH_4Cl to give concentrations ranging from 1 to 75 or 100 μg/ml NH_3 in 50 ml of SSW(1) or (2). Media without ammonia served as controls. 1.0 μg/ml of ammonia was used as a unit because it was estimated to be the mean concentration of ammonia produced in one day by 20 mg snails kept at a density of one snail per 50 ml (Powles, unpublished). Ammonia was also supplied as $(NH_4)_2SO_4$ in some of the experiments in order to ascertain whether the effects observed were similar to those produced by NH_4Cl.

As the relative amounts of ionized and free ammonia are dependent on pH, the effects of varying ammonia concentrations were investigated in media with the

TABLE I

The mean cumulative specific growth rates ($\bar{x}$) achieved by juvenile B. glabrata *fed on Bemax and subjected to various concentrations of ammonia (as NH_4Cl) in SSW(1) buffered at pH 7 and 9 with Tris and Borate buffers respectively. (N = number of snails, s.e. = standard error, *, **, *** differ from controls at P < 0.05, 0.01, 0.001 respectively).*

Buffer used	Duration of experiment in days	Concentration of ammonia in μg/ml											
		0		1		10		25		50		75	
		N	$\bar{x}$ s.e.	N	$\bar{x}$ s.e.	N	$\bar{x}$ s.e.	N	$\bar{x}$ s.e.	N	$\bar{x}$ s.e.	N	$\bar{x}$ s.e.
Tris	0–3	10	0.67 ± 0.01	10	4.33* ± 1.67	10	1.0 ± 0.33	10	1.33 ± 0.33	10	2.33 ± 1.00	10	3.00 ± 2.00
Tris	0–6	10	2.67 ± 0.01	10	6.33* ± 1.33	10	6.5* ± 1.00	10	5.50 ± 1.50	10	7.00* ± 1.33	10	6.17 ± 2.00
Tris	0–9	10	2.89 ± 0.56	10	6.33* ± 1.11	10	6.0** ± 0.89	10	6.22 ± 1.67	10	7.56*** ± 1.00	10	6.22 ± 1.78
Borate	0–3	10	2.33 ± 0.57	10	4.00 ± 1.0	6	3.33 ± 1.33	1					
Borate	0–6	9	3.50 ± 0.89	9	3.00 ± 1.17	5	2.17 ± 0.83	1					
Borate	0–9	0	—	0	—	0	—	0					

following pH values: 5.4–5.5, 7.0, 8.1–8.6, and 9.0. The pH values of 5.4–5.5 and 8.1–8.6 were those of SSW(2) and SSW(1), respectively. Tris and borate buffers were used to achieve pH values of 7 and 9.0 respectively (Dawson, Elliot, Elliott and Jones, 1969). Various other buffers including citrate and glycine buffers were tried and rejected for various reasons including the fact that they formed precipitates on standing in SSW. Both of the buffers used in the present experiments have also been used successfully as buffers in perfusion fluids for invertebrates (Welsh and Smith, 1960).

Assay snails were kept in isolation in 50 ml of nonaerated test or control media. Each treatment was replicated ten times. The experiments which were based on a completely randomized design were subjected to analysis of variance.

Growth was monitored at three day intervals using a weighing method described by Thomas and Benjamin (1974). The growth rate was expressed as the cumulative specific growth rate $(Wt\text{-}Wo) \times 100/Wo \times \Delta t$, where Wo represents weight in mg at time t and Δt is time interval in days.

Results

With the exception of the experimental snails kept in borate buffer SSW(1), the various treatments did not affect the survival rates over the period of the experiment. Table I gives the number of snails placed in borate or tris buffered media surviving to be weighed at the end of each three day interval. As ten snails were used initially in each treatment, it is evident that heavy mortality had occurred in the borate buffered treatments. With one exception all the snails in borate buffers in the 25 and 50 μg/ml treatments had died within three days and by the end of nine days all the remainder had died. It is also evident from Table I that the growth rates of the snails in SSW buffered by both tris and borate had been seriously impaired, as it was only about 10 per cent of that achieved by the control snails in unbuffered SSW (Table II).

It can be seen from Tables I and II, which give the mean cumulative growth rates achieved by the snails in the various treatments that the values for the control snails tend to be rather variable. Factors which influence the growth rate of the snails include their physiological state or growth potential as well as their age and the nature of the medium and the food. It has been found that standardization of the methods of maintaining stock cultures, by using a flow-through system, and of handling procedures has helped to reduce the variability in the growth rates of the snails. Although lettuce is a good quality food which allowed the snails to grow rapidly, it is evident from Table I and II that the standard errors of the mean for lettuce-fed snails tend to be higher than those for snails fed on Bemax. The latter was, therefore, adopted as a food medium in subsequent experiments. The standard errors also tend to increase as the snails become larger. To overcome these problems and to facilitate comparisons between experiments, the mean specific growth rates have been expressed as percentages of the mean control values (Figs. 1–3). Tables I and II show that statistically significant differences between the controls and certain of the treatments may occur as early as the third day. It might be expected that the number of statistically significant treatment effects would increase with time, but this is not necessarily the case in all experiments. In general, however, there is a measure of consistency and the same treatments tend to show statistically significant effects throughout the duration of an experiment.

TABLE II

Mean cumulative specific daily growth rates ($\bar{x}$) achieved by juvenile Biomphalaria glabrata *in the two media SSW(1) and SSW(2) when the concentrations of ammonia were varied from 0–100 μg/ml, P = results of analysis of variance, (*, **, *** differ from controls at P < 0.05, 0.01, 0.001 respectively).*

Treatments				Concentrations of NH_3 in μg/ml							*P* values
Medium	Food source	NH_3 source	Days	0 $\bar{x}\pm$s.e.	1 $\bar{x}\pm$s.e.	10 $\bar{x}\pm$s.e.	25 $\bar{x}\pm$s.e.	50 $\bar{x}\pm$s.e.	75 $\bar{x}\pm$s.e.	100 $\bar{x}\pm$s.e.	
SSW(1)	Lettuce	NH_4Cl	0–3	32.67 ±4.00	36.67 ±6.00	25.67 ±5.67	14.33** ±3.33	13.33** ±3.00	10.67** ±2.67	7.33 ±0.67***	<0.001
SSW(1)	Lettuce	NH_4Cl	0–6	31.67 ±3.67	35.67 ±7.83	24.33 ±4.17	17.67 ±4.67*	17.17 ±5.50*	17.17 ±3.00*	13.83 ±3.17	<0.05
SSW(1)	Lettuce	NH_4Cl	0–9	30.89 ±4.67	40.33 ±7.56	24.89 ±5.56	21.78 ±4.56	20.33 ±5.00	21.44 ±4.11	15.11 ±1.11**	<0.05
SSW(1)	Bemax	NH_4Cl	0–9	48.44 ±3.22	51.11 ±2.67	34.56 ±6.44	27.67 ±3.78***	8.33 ±3.33***	3.78 ±1.89***	0.33 ±0.11***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–3	19.0 ±1.33	16.33 ±1.33	15.33 ±1.67	11.67 ±1.00***	8.0 ±1.00***	2.33 ±1.00***	0.10 ±0.04***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–6	29.67 ±2.50	21.67 ±1.83*	25.33 ±2.67	22.33 ±1.33*	12.0 ±1.50***	4.0 ±1.17***	1.33 ±0.33***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–9	43.44 ±1.89	30.22 ±1.44***	39.22 ±3.78	37.11 ±2.0*	26.67 ±3.22***	11.22 ±2.33***	6.89 ±2.23***	<0.001
SSW(2)	Bemax	NH_4Cl	0–3	24.33 ±1.67	18.67 ±0.67**	13.67 ±1.33***	13.33 ±1.67***	12.33 ±1.33***	9.00 ±0.03***	12.33 ±0.15***	<0.001
SSW(2)	Bemax	NH_4Cl	0–6	24.50 ±2.83	22.00 ±1.33	20.50 ±1.33	21.00 ±1.50	16.67 ±1.50*	15.83 ±1.83*	5.33 ±1.33***	<0.01
SSW(2)	Bemax	NH_4Cl	0–9	32.22 ±3.33	31.11 ±2.67	31.33 ±3.44	19.67 ±1.78**	14.67 ±1.33***	12.11 ±1.56***	20.11 ±2.56**	<0.01
SSW(2)	Bemax	NH_4Cl	0–3	1.33 ±0.33	4.67 ±1.33*	2.33 ±1.00	4.67 ±1.00**	2.67 ±0.67	1.0 ±0.33	1.33 ±0.67	<0.001
SSW(2)	Bemax	NH_4Cl	0–6	9.00 ±0.83	13.00 ±2.50	7.83 ±1.33	11.17 ±1.83	7.17 ±0.50	7.00 ±1.50	3.17 ±1.00***	<0.01
SSW(2)	Bemax	NH_4Cl	0–9	15.67 ±0.89	19.78 ±0.44***	14.56 ±2.11	14.78 ±0.89	10.44 ±1.22**	12.11 ±1.33*	8.33 ±0.56***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–3	21.00 ±1.33	20.0 ±1.67	20.00 ±1.67	23.33 ±1.67	24.67 ±1.00*	22.0 ±2.00	2.0 ±1.33***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–6	24.83 ±2.50	21.33 ±1.33	34.67 ±3.00*	24.0 ±4.33	22.0 ±1.00	22.83 ±1.67	7.0 ±2.83***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–9	27.56 ±2.11	34.44 ±1.67	25.44 ±1.44	22.89 ±1.00	24.00 ±1.33	14.89 ±1.78***	12.0 ±2.33***	<0.001

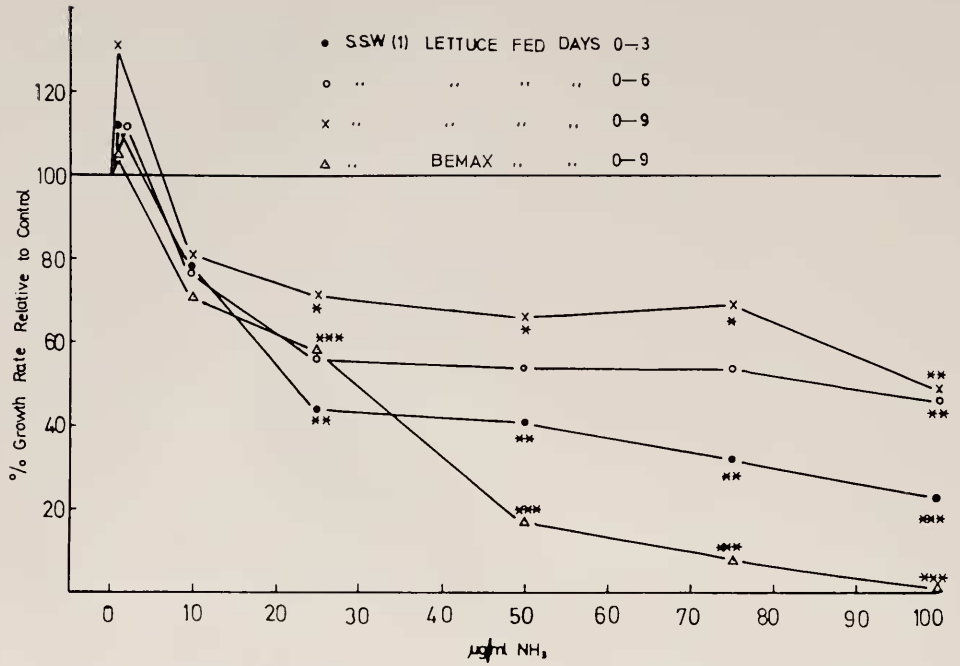

FIGURE 1. Percentage growth rate of snails relative to that of control snails when subjected to various concentrations of ammonia in SSW(1) and fed with either lettuce or bemax.

Analyses of variance undertaken on these data in Tables I and II show that all the treatments, with the exception of those involving buffered media, caused statistically significant treatment effects ($P < 0.01$–0.001). These can be attributed to the fact that the growth rates of the snails tend to be either enhanced or inhibited to a statistically significant extent compared with controls. Enhancement of growth was more commonly encountered in the treatments containing 1 μg/ml of ammonia. In six of these cases the mean cumulative specific growth rates were significantly higher than the controls; three occurred in the tris buffered treatments, two in those in which ammonia was added to SSW (2) as NH_4Cl and one in which it was added as $(NH_4)_2\ SO_4$. Three examples of significant growth enhancemnet also occurred in the treatments receiving 10 μg/ml of ammonia; two occurred in the tris-buffered treatments and one in the chloride medium in which ammonia had been added as ammonium chloride. One case of significant growth enhancement also occurred in each of the treatments receiving 25 and 50 μg/ml of ammonia. It is noteworthy that most of the examples of statistically significant growth enhancement caused by the addition of ammonia occurred in experiments in which the snails were growing relatively slowly.

Growth inhibition also occurred in treatments to which ammonia had been added. The percentage of treatments showing statistically significant growth reduction increased progressively with increase in ammonia concentration above the optimum levels. Thus if the treatments were buffered with borate or tris are

omitted, 18.7, 6.2, 56.2, 68.7, 68.7 and 93.7% of the treatments receiving 1, 10, 25, 50, 75 and 100 μg/ml of ammonia, respectively, show a significant growth reduction. A comparison of Figures 2 and 3 show that the percentage reductions tend to be greater in SSW(1), in which the dominant anion is bicarbonate, than in SSW(2) which has chloride as the dominant anion.

Table III shows that the initial mean pH values are considerably higher in SSW(1) than in SSW(2). However, the pH values of the media decline as a result of conditioning by the snails. It can be seen from Table III that the decrements in pH become progressively greater as the snails become larger. The values in SSW(1) always remain on the alkaline side of neutrality whereas those in SSW(2) reach values as low at 4.6. At any one time the differences between treatments are very slight, although there is some tendency for pH values in SSW(1) to decrease progressively with ammonia concentration by the sixth and ninth days.

The pH values are important because they influence the relative quantities of free ammonia as indicated by the following equation: conc of free ammonia = total ammonia conc/1 + antilog (pKa − pH). If it is assumed that the various media have the following pH values: SSW(2), 6.5; SSW(1) tris-buffered, 7.0; SSW(1), 8.3; and SSW(1) borate-buffered, 9.0 then the calculated values for percentage free ammonia are 0.17, 0.55, 9.82 and 35.46%, respectively. In consequence, the initial concentrations of free ammonia in SSW(2), SSW tris-buffered, SSW(1) and SSW(1) borate-buffered media to which ammonia has been added vary from

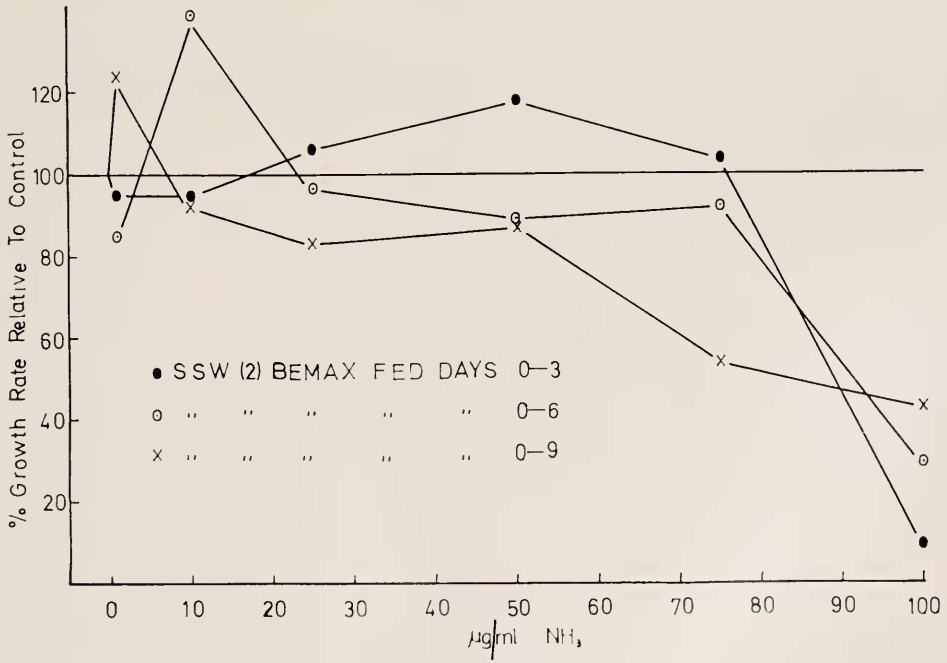

FIGURE 2. Percentage growth rate achieved by snails relative to that of control snails when subjected to various concentrations of ammonia in SSW(2) and fed with bemax.

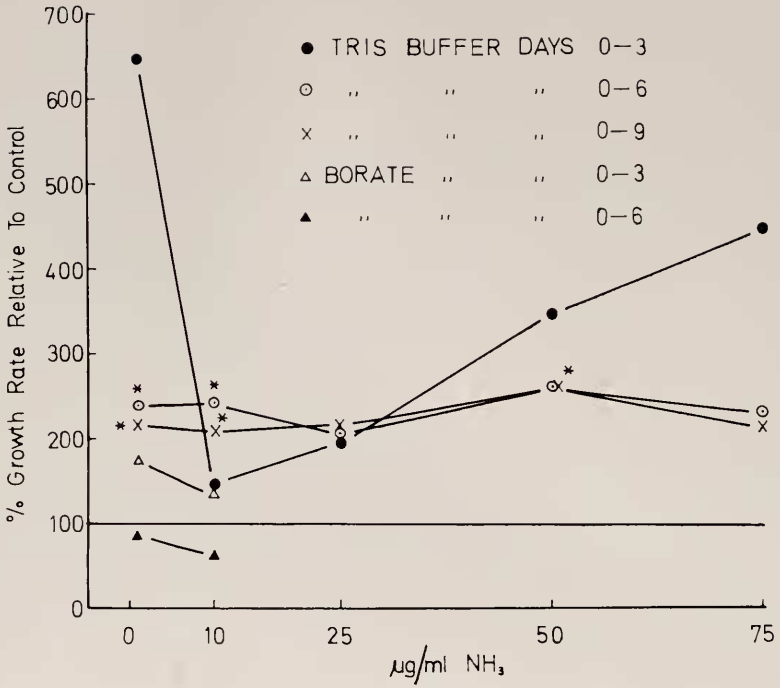

FIGURE 3. Percentage growth rate achieved by snails relative to that of control snails when subjected to various concentrations of ammonia in tris or borate buffers.

1.74×10^{-3} to 1.74×10^{-1}, 5.46×10^{-3} to 5.46×10^{-1}, 9.82×10^{-2} to 9.82 and 3.55×10^{-1} to 35.46 µg/ml respectively.

DISCUSSION

The net movement of ammonia across barriers depends on the partial pressure, P_{NH_3}, on each side of the membranes and occurs from higher to lower tensions. It

TABLE III

The mean pH values in the various treatments at different times. T_0, T_1, T_2 and T_3 correspond with the times when the snails were weighed. The values at time T_0 are therefore the initial values and those at T_1, T_2, T_3 are the values after the snails had conditioned the media for consecutive three day periods.

Medium	Time when measurements taken	Concentration (µg/ml NH_3)						
		0	1	10	25	50	75	100
SSW(1)	T_0	8.6	8.5	8.5	8.4	8.3	8.2	8.1
	T_1	8.7	8.6	8.4	8.3	8.2	8.1	8.0
	T_2	7.5	7.5	7.6	7.6	7.8	7.9	7.8
	T_3	7.2	7.2	7.3	7.5	7.6	7.9	7.8
SSW(2)	T_0	6.5	6.4	6.3	6.3	6.2	6.2	6.2
	T_1	5.5	5.5	5.6	5.5	5.4	5.4	5.4
	T_2	4.7	4.7	4.7	4.7	4.6	4.6	4.6
	T_3	4.6	4.6	4.6	4.6	4.6	4.6	4.6

has been shown that P_{NH_3} is proportional to pH: $pH = pK + \log NH_3/NH_4$ (Maetz, 1972). Free ammonia will, therefore, pass from the side of higher to lower pH. As free ammonia diffuses readily across membranes because of its high lipid solubility and the ambient pH of 9.0 in the borate medium is probably higher than that of the internal environment, it is to be expected that outward diffusion would be prevented and inward diffusion facilitated. This hypothesis is supported by observations which indicate that the pH of the pallial fluid of molluscs varies from 7.7–8.4 (Stolkowski, 1951) and molluscan blood from 7.4–7.6 (Speeg and Campbell, 1968). The internal ammonia concentrations of snails in media buffered by borate may, therefore, rapidly reach toxic proportions even in the control treatments to which ammonia had not been added.

It has been shown that snails alter the chemical composition of the medium in which they live by taking up ions such as Na^+, Ca^{++} and Fe^{+++} and by releasing other such as NH_4^+, H^+ and HCo_3^- (Thomas, Goldsworthy and Aram, 1975; Thomas and Aram, 1974). Recent work (Maetz, 1972) has shown that the release of endogenous ions such as NH_4^+ and HCO_3^- by aquatic animals including snails may be tightly coupled with the uptake of exogenous ions such as Na^+ and Cl^-, respectively. It is possible, therefore, that the buffered media interfere with the coupled exchange of ions. An alternative explanation is that both 'tris' and borate ions are taken up by the snails and have an adverse effect on their physiology. Tris-buffered solutions may also be harmful because they absorb CO_2 from the air (Dawson *et al.*, 1969).

Since free ammonia passes from the side of higher to lower pH, (Maetz 1972) and the pH of the pallial fluid in molluscs is 7.7–8.4, (Stolkowski, 1951) and that of molluscan blood 7.4–7.6 (Speeg and Campbell, 1968), it is possible that *B. glabrata* may take up free ammonia from SSW(1) and borate-buffered media as their pH's are 8.3–8.5 and 9.0, respectively. It is unlikely, however, that free ammonia can enter the snail from the tris-buffered media and SSW(2), as the pH values are 7.0 and < 7.0, respectively. On the other hand, there is a possibility that even in these two media the snails may take up NH_4^+ from the medium by active transport (Maetz, 1972). According to Fromm and Gillette (1968), there is a direct linear correlation between the concentration of ambient ammonia and that of blood ammonia in aquatic animals, including fish, when they are exposed to varying concentrations of ammonia. The snails may, therefore, take up ammonia from the medium in both the dissociated and undissociated form.

As free ammonia is a proton acceptor ($NH_3 + H^+ = NH_4^+$), once it enters the snail it may become involved in several physiological processes which are beneficial and result in growth enhancement. First, ammonia may help to maintain the pH of the blood. Carbonic anhydrase in the mantle tissue catalyzes the production of HCO_3^- and H^+ according to the following reaction: $CO_2 + H_2O = HCO_3^- + H^+$. If the proton is then captured by NH_3, the mantle tissues may act as a diffusion trip for the blood CO_2 and help to control the CO_2 concentration and pH of the blood. The involvement of ammonia in controlling the pH of fish blood has been demonstrated experimentally by Wolbach, Heinemann and Fishman (1959).

Secondly, free ammonia may also facilitate the deposition of $CaCO_3$ in areas of shell growth. According to Berner (1968), Speeg and Campbell (1969), and Campbell and Bishop (1970), there are good reasons for believing that ammonia may function in the deposition of calcium carbonate *via* the following reaction:

$NH_3 + HCO_3^- + Ca^+ = CaCO_3 + NH_4^+$. This has recently been tested as a model for the geochemical deposition of $CaCO_3$ (Berner, 1968).

Other mechanisms that may be facilitated by ammonia include arginine metabolism (Castaneda, Martuscelli and Mora, 1967), and the uptake of ions. It has been suggested by Maetz (1972) that NH_4^+ may be involved in the uptake of Na^+ from the medium by means of a tightly coupled exchange mechanism.

In view of the potential importance of ammonia to snails, it has been suggested by Speeg and Campbell (1969) that they may be able to control its release by synthesizing urea which is then broken down by urease. This enzyme has been shown to occur in many snails (Florkin, 1966; Hammen, Hanlon and Lum, 1962; Hammen, Miller and Geer, 1966; Speeg and Campbell, 1969). The latter authors have also produced other evidence in support of their theory, including the apparent involvement of arginine and arginase. They argue that since urea synthesis, *de novo,* starts with NH_4^+ either directly as NH_4^+ or as a glutamine and HCO_3^- and requires an expenditure of at least 2 moles of ATP it is difficult to envisage it simply as an excretory mechanism. Purine and purine nucleoside deaminases are also present in molluscs (Campbell, Drotman, McDonald and Tramell, 1972). However, because *B. glabrata* is predominantly a herbivore, it can be postulated that its requirements for detoxifying excess ammonia from protein catabolism may be minimal and it is possible that purine biosynthesis may suffice (Campbell, et al, 1972). It can be suggested, therefore, that in view of the physiological importance of ammonia to the snail, it will be selectively advantageous for them to be able to take up ambient ammonia when necessary. It is possible that this source of ammonia may be particularly important to the slow growing snails.

Biomphalaria glabrata seems able to withstand higher levels of ambient ammonia than many other aquatic organisms. Kawomoto, (1961) found that 0.3 μg/ml NH_4Cl inhibited growth of carp, while Burrows (1964) claims that concentrations of NH_4OH varying from 0.3–0.7 μg/ml (0.006–0.018 μg/ml NH_3) caused hyperplasia in the gill filaments of salmon parr. According to Ball (1967) the asymptotic LC_{50} values for perch and trout were 0.29 and 0.41 μg/ml N of undissociated ammonia. In the present investigation it was found that the survival of the snails was not affected except in the media buffered by borate. The growth of the snails was not often inhibited until the total concentration of ammonia was 25 μg/ml. This is equivalent to a concentration of 2.455 μg/ml and 0.0435 μg/ml of free ammonia in the SSW(1) and SSW(2), respectively. Molluscs also generally have higher concentrations of ammonia in their blood compared with other organisms. Thus, species of *Helix, Otala, Anodonta,* and *Sepia* have 7.20, 3.6, 0.51–0.71 and 28–48 μg/ml of ammonia compared with < 1.0 μg/ml in amphibia and 0.1–0.3 μg/ml in mammals (Prosser and Brown, 1962; Speeg and Campbell, 1968).

The growth inhibitory effect may be caused in two ways. First, the ammonium ion in the external medium may compete with ions such as sodium and possibly calcium for available transport sites (Shaw, 1960; Maetz, 1972). Secondly, excess NH_3 and NH_4 may cause toxic effects at the cellular level by interfering with the process of proton translocation across mitochondrial membranes which occur during phosphorlylation (Campbell *et al.,* 1972). According to Spotte (1970) and Maetz (1972), most of the studies concerning toxicity of ammonia in ambient water indicate that the toxicity of any given ammonia solution is augmented when the external pH is elevated due to the fact that the concentration of free ammonia

increases with pH. The present study provides some support for this hypothesis because the inhibitory effects have the following sequence: borate buffered medium > SSW(1), > SSW(2). Apparently, however, there are cases where toxicity of ammonia is augmented by a decrease in pH when the latter is caused by free carbon dioxide (Maetz, 1972).

One important question is whether exogenous ammonia produced by snails could provide a mechanism for controlling growth at the individual or population level of organization in closed systems, under experimental conditions. Previous investigations (Thomas, Benjamin and Lodge, unpublished) have shown that after three days of conditioning by *B. glabrata* in the 100, 300, 500 and 700 mg weight categories, kept at densities of eight snails per 200 ml of SSW(1) (25 ml per snail), the mean concentrations of ammonia were 9.3, 8.5, 13.5 and 6.8 μg/ml NH_3 respectively. The results of the present investigation indicate that these concentrations would only exert a weak inhibitory effect. The fact that the snails also release hydrogen ions into the medium during the conditioning process and thus help to detoxify the free ammonia, also militates against the hypothesis that ammonia provides the basis of a negative feedback mechanism, except possibly when snails are kept in SSW(1) or SSW(2) under very high density conditions for long periods.

The other environmental factors that may be involved in inducing the negative feedback effects observed when snails are kept under sub-optimal density or volume conditions have been discussed by Thomas (1973) and Thomas, Lough and Lodge (1975). Under certain circumstances these effects may be caused by depletion of resources including oxygen, food and ions such as calcium or iron as well as by substances released into the medium by the snails, their food supply or by microorganisms. Thomas, Lough and Lodge (1975) concluded that although the snails appeared to produce factors which enhance growth there is no evidence that they also produce specific factors, which have a detrimental effect on growth, reproduction or survival like those which Berrie and Visser (1963) and Rose and Rose (1961) claimed to have demonstrated in media containing molluscs and tadpoles respectively. Further work is required before a full understanding of the negative feedback mechanisms involved in regulating snail populations can be achieved.

In the natural environment, although ammonia is a major excretory product of other aquatic organisms as well as snails and is also a product of bacterial decomposition, its concentration tends to be low because of the rapidity with which it is oxidized by bacteria, assimilated by plants or lost by diffusion into the air. Thus, Schutte and Frank (1964) state that only very small amounts of ammonia were found occasionally in freshwater bodies in the Transvaal. This also appears to be the case in other freshwaters in Africa (Talling and Talling, 1965) and in the temperate regions of the world (Hutchinson, 1957). According to Talling and Talling (1965) the distribution of ammonia nitrogen in the surface water of African lakes is probably less than 0.040 μg/ml although much larger amounts are detectable in the deoxygenated, lower layers of stratified lakes. One of the highest concentrations of ammonia recorded by Hutchinson (1957) was 0.168–0.544 μg/ml NH_3N in Lake Manona when it was subjected to sewage contamination. It can be concluded, therefore, that except perhaps for snails living under crowded

conditions in closed, alkaline waters, ammonia is unlikely to be a major factor limiting the growth of the snails in nature.

We wish to thank the Overseas Development Administration, WHO Geneva and Shell Research Ltd., for financial help and Mrs. W. Cocks and Mr. A. Lough for assistance and their interest in the work.

Summary

When juvenile specimens of *Biomphalaria glabrata* were subjected to concentrations of ammonia ranging from 1–100 μg/ml in various media the following effects were observed: the addition of ammonia to borate buffered media caused mortality. Both borate and tris-buffered media caused a decrease in the growth rate of snails when compared with controls in SSW. The growth rates of the snails could be enhanced by increasing the concentration of ammonia to critical thresholds, but further increases beyond these thresholds resulted in growth inhibition. The toxicity of ammonia in ambient water was augmented by an an increase in pH. The possible causation and ecological significance of these effects are discussed. There are indications that the snails are physiologically well-adapted to utilize ammonia when required and also to control its excretion and uptake from the medium.

LITERATURE CITED

Ball, I. R., 1967. The relative susceptibilities of some species of freshwater fish to poisons: I. Ammonia. *Water Res.*, **1**: 767–775.

Berner, R. A., 1968. Calcium carbonate secretions formed by the decomposition of organic matter. *Science*, **159**: 195–197.

Berrie, A. D. and S. A. Visser, 1963. Investigations of a growth-inhibiting substance affecting a natural population of freshwater snails. *Physiol. Zool.*, **36**: 167–173.

Burrows, R. E., 1964. Effects of accumulated excretory products on hatchery reared salmonids. *U. S. Bur. Sport Fish. Wildlife Resour. Publ.*, **66**: 1–12.

Campbell, J. W. and S. H. Bishop, 1970. Nitrogen metabolism in molluscs. Pages 103–256 in J. W. Campbell, Ed., *The invertbrata: comparative biochemistry of nitrogen metabolism*. Academic Press, London and New York.

Campbell, J. W., R. D. Drotman, J. A. McDonald and P. R. Tramell, 1972. Nitrogen metabolism in terrestrial invertebrates. Pages 1–54 in J. W. Campbell and L. Goldstein, Eds., *Nitrogen metabolism and the environment*. Academic Press, London and New York.

Castaneda, M., J. Martuscelli, and J. Mora, 1967. The catabolism of *L. arginine* by *Neurospora crassa*. *Biochem. Biophys. Acta*, **141**: 276–286.

Dawson, R. M. C., D. C. Elliott, W. H. Elliott and K. M. Jones, 1969. *Data for biochemical research*, Clarendon Press, Oxford, 654 pp.

Florkin, M., 1966. Nitrogen metabolism. Pages 309–351 in Wilbur and Yonge, Eds., *Physiology of Mollusca II*. Academic Press, New York and London.

Fromm, P. O. and J. R. Gillette, 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.*, **26**: 887–896.

Hammen, C. S., D. P. Hanlon and S. C. Lum, 1962. Oxidative metabolism of *Lingula*. *Comp. Biochem. Physiol.*, **5**: 185–191.

Hammen, C. S., H. F. Miller Jr. and W. M. H. Geer, 1966. Nitrogen excretion of *Crassostrea virginica*. *Comp. Biochem. Physiol.*, **17**: 1199–1200.

Hutchinson, G. E., 1957. *A treatise in limnology: I. Geography, physics and chemistry*. John Wiley and Sons, New York, 1015 pp.

Kawamoto, N. Y., 1961. The influence of excretory substances of fish on their own growth. *Progr. Fish Cult.*, **23**: 70–75.

Maetz, J. 1972. Interaction of salt and ammonia transport in aquatic organisms. Pages 105–154 in J. W. Campbell and L. Goldstein, Eds., *Nitrogen metabolism and the environment.* Academic Press, London.

Prosser, C. L. and J. A. Brown, 1962. *Comparative animal physiology,* Saunders Philadelphia, London, 688 pp.

Rose, S. M. and F. C. Rose, 1961. Growth-controlling exudates of tadpoles. *Symp. Soc. Exp. Biol.,* **15**: 207–218.

Schutte, C. H. J. and G. H. Frank, 1964. Observations on the distribution of freshwater molluscs and chemistry of the natural waters in the south eastern Transvaal and adjacent northern Swaziland. *Bull. World Health Organ.,* **30**: 389–400.

Shaw, J., 1960. The absorption of sodium ions by the crayfish *Astacus pallipes herebouller III.* The effect of other cations in the external solution. *J. Exp. Biol.,* **37**: 548–556.

Speeg, K. V. Jr. and J. W. Campbell, 1968. Formation and volatilization of ammonia gas by terrestrial snails. *Amer. J. Physiol.,* **214**: 1392–1402.

Speeg, K. V. Jr. and J. W. Campbell, 1969. Arginine and urea metabolism in terrestrial snails. *Amer. J. Physiol.,* **216**: 1003–1012.

Spotte, S. H., 1970. *Fish and invertebrate culture.* Wiley-Interscience, New York, 145 pp.

Stolkowski, J., 1951. Essai sur le determinisme des formes minerologique du calcaire chez les êtres vivants (calcaires coquilliers). *Ann. Inst. Oceanogr. Paris,* **26**: 1–113.

Talling, J. F. and I. B. Talling, 1965. The chemical composition of African lakewater. *Int. Rev. Ges. Hydrobiol.,* **50**: 421–463.

Thomas, J. D., 1973. Schistosomiasis and the control of molluscan hosts of human schistosomes with particular reference to possible self regulatory mechanisms. Pages 387–394 in B. Dawes, Ed., *Advances in parasitology, Vol. II.* Academic Press, London, New York.

Thomas, J. D. and R. H. Aram, 1974. The chemical ecology of *Biomphalaria glabrata* (Say). The effects of media homotypically conditioned by adult snails on the growth of juveniles. *J. Exp. Zool.,* **190**: 329–334.

Thomas, J. D. and M. Benjamin, 1974. The effects of population density on growth and reproduction of *Biomphalaria glabrata,* (Say) (Gasteropoda: Pulmonata). *J. Anim. Ecol.,* **43**: 31–50.

Thomas, J. D., G. J. Goldsworthy and R. H. Aram, 1975. Studies on the chemical ecology of snails: the effects of chemical conditioning by adult snails on the growth of juvenile snails of the same species. *J. Anim. Ecol.,* **44**: 1–27.

Thomas, J. D., A. S. Lough and R. W. Lodge, 1975. The chemical ecology of *Biomphalaria glabrata* (Say) the snail host of *Schistosomo mansomi* Sambon: the search for factors in media conditioned by snails which inhibit their growth and reproduction. *J. Appl. Ecol.,* **12**: 421–436.

Welsh, J. H., and R. I. Smith, 1960. *Laboratory exercises in invertebrate physiology.* Burgess Publ. Co., Minneapolis, 179 pp.

Wolbach, R. A., H. O. Heinemann and A. D. Fishman, 1959. Fate of intraperitoneally injected ammonia in the catfish, *Ameirus nebulosus.. Bull. Mt. Desert Island Biol. Lab.,* **4**: 56–57.